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TITLE: Expression Profiling of Cell Lines Expressing Regulated NF2 Transcripts

PRINCIPAL INVESTIGATOR: Stefan M. Pulst, M.D.

CONTRACTING ORGANIZATION: Cedars-Sinai Medical Center
Los Angeles, California 90048

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6. AUTHOR(S):
Stefan M. Pulst, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Cedars-Sinai Medical Center
Los Angeles, California 90048

E-Mail: Pulst@CSHS.org

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13. ABSTRACT *(Maximum 200 Words)*Expression profiling is a powerful novel technique to examine changes in the expression of a large number of genes at the same time. Parallel analysis of gene expression reflects the changing view of signaling pathways towards signaling networks that have multiple and complex feedback and feed forward loops. Different phenotypic states of a cell can be translated into specific gene expression signatures. Expression profiling is not restricted to known genes, but changes in the expression of genes without known function can also be detected. As a complement to yeast two-hybrid studies we proposed using gene expression profiling to determine changes in gene expression as a function of NF2 expression in schwannoma cells. The strength of our approach is that we will not use tissues from patients, but will concentrate on cell lines in which NF2 expression can be controlled through the Tet/On system. We have now generated several cell lines that express NF2 in a regulated fashion. The parent lines are RT4 schwannoma cells and mouse embryonic fibroblasts. A time course for NF2 expression has been established. A total of four cell lines have been tested on microarrays to detect expression changes. Surprisingly, no changes common to expression of isoform 1 and 2 have been detected so far. We are currently in the process of repeating the experiments with slightly altered conditions to enhance the reliability of the detected expression changes.

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Introduction:

The NF2 protein, also called schwannomin or merlin, is a member of the ezrin-radixin-moesin family of proteins. Based on mutation analysis and protein expression studies, it is thought that NF2 is a tumor suppressor gene and that biallelic inactivation is required for phenotypic expression. Studies in cells that are NF2 deficient or that overexpress NF2 cDNAs have shown a powerful role of NF2 in regulation of cell morphology and proliferation. Despite these advances little is known about the action of NF2 in specific signaling pathways. This knowledge is of importance because it may lead the way to identifying treatments that modify these pathways.

Expression profiling is a powerful novel technique to examine changes in the expression of a large number of genes at the same time. Parallel analysis of gene expression reflects the changing view of signaling pathways towards signaling networks that have multiple and complex feedback and feed forward loops. Different phenotypic states of a cell can be translated into specific gene expression signatures. Expression profiling is not restricted to known genes, but changes in the expression of genes without known function can also be detected.

We are proposing to use gene expression profiling to determine changes in gene expression following regulated expression of NF2 cDNAs in schwannoma and meningioma cells. We will test the following hypotheses: 1. Regulated expression of NF2 results in specific changes in the expression profile at specific time points. 2. A subset of regulated transcripts differs between cells expressing NF2 isoform 1 and NF2 isoform 2. 3. NF2 induction results in partially shared expression changes in different cell types. 4. Expression changes are context dependent; expression profiles in confluent cells are different from non-confluent cells.

Body

Statement of Work Year 1

In the first year we will begin with the analysis of timed NF2 expression. In the second half of the first year we will examine the effects of overexpressing NF2 isoform 1 or isoform 2 in rat RT4 schwannoma cells.

For those genes that show consistent changes in expression we will perform quantitative northern blot analysis to validate expression changes.

We will continue the generation of meningioma cells. Lines expressing wildtype and mutant forms of NF2 will be generated in the Tet/On system.

We have established parameters for the induction of NF2 expression in RT4 cells. We had problems generating meningioma cells that stably express NF2. We therefore used mouse embryonic fibroblasts (MEF). cDNAs encoding NF2 isoforms 1 and 2 were put under the control of the tet-responsive promoter and introduced into MEF/3T3 TET-OFF cells using retroviral transduction.

These cells show a robust induction of NF2 expression at the RNA and protein levels after removal of doxycycline.

We have purified RNAs from RT4 and MEF cells and used these for expression profiling with the Affymetrix MG_U74Av2 chips. At a significance level of 0.05, we found that approximately 50 genes were regulated upon NF2 expression. This was true for either isoform.

Interestingly, no regulated genes were shared between isoform 1 and 2.

Key Research Accomplishments

- Establishment of cell lines that express NF2 under control of the TET-regulator.
- Establishment of array profiles for both NF2 isoforms.

Reportable Outcomes

Manuscripts in preparation: Oh MK & Pulst SM: Genetic heterogeneity of stably transfected cell lines revealed by expression profiling with oligonucleotide arrays.

Presentations

Oh MK & Pulst SM: Expression profiling for the analysis of NF2 function. UCLA Array Symposium, 2002.

Conclusions

Gene expression profiling is still in its infancy. We are beginning to answer some basic questions regarding the use of stably transfected cell lines and the role of schwannomin and HRS. These results will provide a framework to evaluate the alterations seen in human tumors associated with NF2 mutation.